

Isolated limb perfusion: a therapeutic option for nodular and acral lentiginous melanoma

Perfusión aislada de extremidad: una opción terapéutica para metástasis en tránsito de melanoma nodular y lentiginoso acral

Carlos Duarte¹, Juliana Restrepo^{2*}, Alden Pool-Gómez³ and Alexander Carreño⁴

¹Department of Breast and Soft Tissue, Instituto Nacional de Cancerología, ESE, Asociación Colombiana de Mastología, Universidad del Rosario, Universidad Militar Nueva Granada, Bogotá; ²Department of Surgical Oncology, Instituto Nacional de Cancerología, ESE, Universidad Militar Nueva Granada, Bogotá; ³Department of Surgical Oncology, Hospital Universitario del Valle, Universidad del Valle, Cali; ⁴Clinical Research Group, Instituto Nacional de Cancerología, ESE, Bogotá, Colombia

Abstract

Objective: to describe the initial experience in Colombia of locally advanced melanoma treated by isolated limb perfusion and compare the response rate of our melanoma subtypes with the literature. **Methods:** a descriptive, retrospective study of a series of patients selected between 2007 and 2016. Qualitative variables were analyzed with proportions and frequencies, and quantitative variable with central tendency measures and measures of dispersion. **Results:** 11 patients were treated by isolated limb perfusion. After the treatment, seven patients (63.6%) had partial response, two patients (18.2%) had stable disease and two patients (18.2%) had disease progression. Five patients (45.5%) had mild complications. **Conclusions:** Isolated limb perfusion is still an important treatment strategy for locally advanced melanoma, with the goal of improving the patient quality of life. The response can change according to the type of melanoma, and could be smaller in patients with acral lentiginous and nodular melanoma, although we cannot make this conclusion with this type of study because it has low power and lack of a comparison group.

Keywords: Melanoma. Chemotherapy. Cancer. Regional perfusion. Limb salvage.

Resumen

Objetivo: describir la experiencia inicial en Colombia en el manejo del melanoma de extremidad con perfusión aislada y comparar la respuesta de los subtipos histológicos más frecuentes en Colombia con la literatura. **Materiales y métodos:** estudio descriptivo, retrospectivo de una serie de pacientes con diagnóstico de melanoma con metástasis en tránsito tratados con perfusión aislada de extremidad, seleccionados entre 2007 y 2016. Las variables cualitativas se analizaron con frecuencias absolutas y relativas. Las cuantitativas con medidas de tendencia central y dispersión. **Resultados:** 11 pacientes fueron tratados con perfusión aislada de extremidad. Hubo respuesta parcial en siete pacientes (63,6%), enfermedad estable en dos pacientes (18,2%) y progresión en dos pacientes (18,2%). Después del tratamiento, cinco pacientes (45,5%) presentaron complicaciones menores. **Conclusiones:** la perfusión aislada sigue siendo importante para el tratamiento del melanoma localmente avanzado con el fin de mejorar la calidad de vida de los pacientes. La respuesta puede cambiar según el tipo clínico de melanoma, siendo menor, según este estudio, en pacientes con melanoma lentiginoso acral y nodular, aunque por el bajo poder, esto no se puede concluir.

Palabras clave: Melanoma. Quimioterapia. Cáncer. Perfusión regional. Salvamento de extremidad.

*Correspondence:

Juliana Restrepo
Calle 1 No. 9-85
C.P. 110411, Bogotá, Colombia
E-mail: julirpo85@gmail.com

Date of reception: 25-05-2020
Date of acceptance: 19-10-2020
DOI: 10.24875/CIRUE.M20000348

Cir Cir (Eng). 2021;89(4):458-464
Contents available at PubMed
www.cirurgiaycirujanos.com

2444-0507/© 2020 Academia Mexicana de Cirugía. Published by Permanyer. This is an open access article under the terms of the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Isolated limb perfusion (ILP) is an alternative treatment in patients with locally advanced melanoma of the extremities, in which high concentrations of chemotherapeutic drugs are obtained in the compromised limb. Its main goal is to decrease tumor burden and preserve the limb. Systemic toxicity should be avoided by using a rigorous surgical technique¹⁻³.

Although there are centers with more than 50-year experience on this treatment in the world^{4,5}, there are few studies reporting the experience in Latin America⁶. In some countries such as Colombia, the incidence of certain subtypes of melanoma such as acral lentiginous is higher and, therefore, isolated limb perfusion response may have a different behavior, when compared with the results in countries with predominance of other clinical subtypes.

The present study describes ILP results in patients with melanoma with in-transit metastasis (ITM) treated at the National Cancerology Institute (INC – *Instituto Nacional de Cancerología*) in Bogotá, Colombia. The purpose of the study is to describe our initial experience and compare the results with other countries in Latin America and the world.

Methodology

Descriptive, retrospective study in which an active search on INC electronic medical records system (MRS) was carried out. Patients diagnosed with melanoma of the limb with ITM treated with ILP between January 2007 and January 2016 were selected. Identified cases had to meet the following inclusion criteria: melanoma histopathological confirmation, melanoma of any clinical subtype with ITM, and having received ILP treatment with curative or palliative intent.

The sociodemographic age and gender variables were obtained from each medical record. Clinical variables included location, melanoma clinical subtype, Breslow's depth, lymph node status, and clinical stage at diagnosis. Outcome variables of the procedure included operating time, hospital and intensive care unit (ICU) stay, complications, and response to treatment according to World Health Organization criteria (Complete Response, Partial Response, Stable Disease and Disease Progression). Data on treatment toxicity were also obtained, which was classified according to the Wieberdink scale⁷.

Data were collected in Excel (Microsoft Corp) and analyzed using SPSS 22 according to the variables' nature; for qualitative variables, an analysis was carried out for proportions and frequencies; as for quantitative variables, whether their distribution was normal was determined, and were analyzed as appropriate in terms of central tendency and dispersion measures.

Approval was obtained from the ethics committee of the National Institute of Cancerology.

Description of the technique: isolated limb perfusion

All patients received ILP treatment under general anesthesia. Invasive monitoring, central venous catheter and arterial line were used. Initially, the main arteries and veins of the affected limb proximal to the tumor were dissected. Dissection is performed in a 5-cm trajectory, ligating all collateral vessels, and the limb is isolated with a pneumatic tourniquet, at a pressure of 250 mmHg. Subsequently, the main artery and vein were catheterized. In the upper limb, the anatomical locations susceptible to intervention are the axillary or humeral vessels, and in the lower limb, the iliac, common femoral, superficial femoral, and popliteal vessels. Arteriotomy and venotomy were carried out transversely in order to reduce the risk of stenosis. The artery and vein were channeled by means of cannulas, into an extracorporeal circulation circuit that includes an oxygenator and a heater that maintains the system at 41 °C.

Pyrophosphates were injected into the circuit, which have the purpose to sensitize red blood cells to technetium-99, which will be subsequently infused. A gamma probe in the precordial region ruled out leakage into general circulation, and Melphalan was administered at a dose of 10 mg/l of perfused limb for lower limb lesions and 13 mg/l for upper limb lesions. Melphalan concentration is 10 to 20 times higher in the isolated circuit in comparison with the usual systemic dose. Isolated infusion was carried out for 60 minutes, the catheters were removed, and the vessels were repaired.

Results

Eleven patients were included; median age was 59 years (range: 49 to 67 years), eight (72.7%) were women, and in nine cases (81.8%), the compromise was in lower limbs. In the classification by clinical

subtypes, seven patients (63.6%) had acral lentiginous melanoma and four (36.4%) had nodular melanoma. Median Breslow depth (range) was 3.6 mm (1.1-20), and 8 patients (72.7%) had positive lymph nodes and in-transit metastases at diagnosis (Table 1).

Median operative time (range) was 267.5 (105-375) minutes, median days of hospital stay (range) was 6.5 (1-17) days, and median ICU length of stay (range) was 2 (0-5) days (Table 2).

With isolated limb perfusion, partial response was obtained in seven patients (63.6%), stable disease in two patients (18.2%), and disease progression in two patients (18.2%) (Table 2).

Median survival was 17 months, the probability of survival at 1 year was 70.7%, and at 33 months was 36.4% (Fig. 1). Four patients (36.4%) relapsed (two men and two women) and nine patients (81.8%) died at an average follow-up of 18 months (range: 3 to 30 months) (Table 2).

Complications after ILP were classified according to Wieberdink⁷ (Table 2). Mild edema was observed in eight patients (72.7%), and moderate edema in one (9.1%). Late postsurgical complications included two cases (18.2%) of surgical site infection and one case of deep vein thrombosis (9.1%). From the technical point of view, there was antineoplastic agent leakage in one procedure (9.1%). (Table 2). There were no cases of severe systemic toxicity or mortality associated with the procedure.

Discussion

Globally, the incidence of melanoma continues to increase⁸. In Latin America, epidemiological information on melanoma is limited and only institutional experiences are described⁹⁻¹². An epidemiological review published in Brazil in 2011 found that the incidence is lower than that in more developed regions such as Europe and North America¹³.

In Colombia, some studies have shown a relative incidence increase, and the predominant clinical pattern is acral lentiginous^{9,14}. In a study published in 2018 by a private institution in Colombia¹², superficial spreading melanoma accounted for 42%, lentigo maligna for 31% and acral lentiginous melanoma for 21%. Unlike a previous study published by the National Institute of Cancerology⁹, a public institution, where the population is similar to the Colombian general population and where the predominant pattern was acral lentiginous, which accounted for 45% of the series. This can be explained by the fact that the series

Table 1. Patient demographic and clinical characteristics

Variables	Description	n	Number (%) or median (range)
Gender	Males	3	27.3
	Females	8	72.7
Average age (range)	59	11	49-67
Location	Upper limb	2	18.2
	Lower limb	9	81.8
Classification	Acral lentiginous	7	64
	Nodular	4	36
Breslow depth at diagnosis	Millimeters	11	3.6 (1.1-20)
Lymph node status at diagnosis	Negative	3	27.3
	Positive	8	72.7
Clinical stage at diagnosis	I	1	9.1
	II	2	18.2
	III	8	54.5

*According to AJCC staging manual, 7th ed.

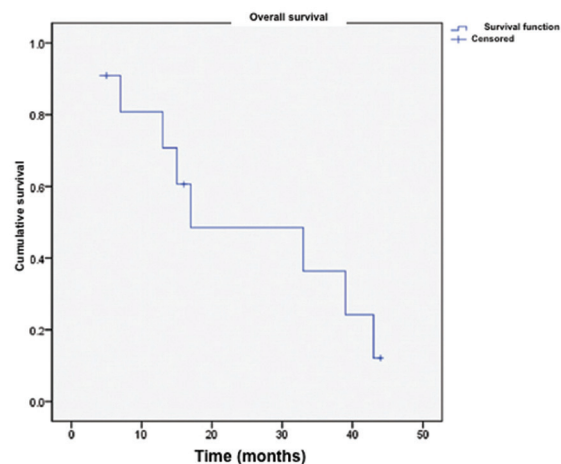


Figure 1. Kaplan-Meier chart describing the survival of the cohort of patients with melanoma in-transit metastases treated with ILP. ILP: Isolated Limb Perfusion.

published in 2018 has a predominance of Fitzpatrick I to III phototypes, which are phototypes that predominate in high-income people who use private health systems.

In Anglo-Saxon studies, the superficial spreading growth pattern predominates⁸, while in our institution, acral lentiginous melanoma does⁹, and, for this reason, we consider it important to know ILP results in

Table 2. Safety of the procedure and treatment response

Variable	Description	n	Number (%)/ median (range)
Surgical time	Minutes	11	267.5 (105-375)
Hospital stay	Days	11	6.5 (1-17)
Intensive Care Unit	Days	11	2 (0-5)
Perfusion failure	No	9	81.8
	Yes	2	18.2
Complications during ILP	Agent leakage	1	9.1
Infusion toxicity according to Wieberdink scale	I	2	18.2
	II	8	72.7
	III	1	9.1
	IV	0	0.0
	V	0	0.0
Postsurgical complications	Superficial SSI*	2	18.2
	Deep SSI*	1	9.1
	Neurological deficit	1	9.1
Systemic complications	Hydroelectrolytic disorder	1	9.1
	Lung disorders	1	9.1
Infusion response	Partial	7	63.6
	Complete	0	0.0
	Stable	2	18.2
	Progression	2	18.2
Treatment response	Relapse	4	36.4
	Limb salvage	11	100
18-month survival	Alive	2	18.2
	Dead	9	81.8

SSI: surgical site infection.

the INC population, where the acral lentiginous clinical pattern predominates.

In Latin America, the first report on ILP for the treatment of melanoma was published in Brazil in 2008; 41 patients were included, with a complete response being obtained in 43% and partial response in 36%, but there is no description of clinical subtypes distribution⁶. In 2007, this technique was implemented for the first time in Colombia for the management of tumors on extremities, and in a publication of 2011, the results of patients diagnosed with melanoma, sarcoma and cutaneous adnexal carcinoma were reported¹⁵.

In patients diagnosed with melanoma, in-transit metastases occur at advanced stages of the disease and are considered a clinical condition of poor prognosis. From the therapeutic point of view, in-transit metastases surgical resection is a treatment that is able to improve symptoms and impaired function of the compromised limb¹, but some patients develop multiple lesions or large lesions that are not susceptible to surgical resection¹. In patients with locally advanced, unresectable limb melanoma, ILP has multiple studies on its effectiveness and toxicity¹; this treatment can avoid amputation and improve the quality of life of patients with this pathology. The choice of therapy

depends on the number of lesions, their anatomical location and size, and the presence or absence of extra-regional disease^{16,17}. Amputation is almost never indicated and does not improve survival in these patients¹⁸.

In this study, response is lower than that reported in the literature, with partial response in two-thirds of patients and stable disease in 18.2%. Different studies have published response rates of between 80 and 90%^{1,19} and complete response reports are around 50%^{3,19,20}. These publications are heterogeneous with regard to tumor burden, and the distribution of clinical subtypes varies according to demographic location, which could explain the different response rates. Even so, the response continues to be higher than the response of metastatic melanoma to systemic cytotoxic chemotherapy, which ranges from 15 to 46%, without overall survival being impacted^{20,21}. A study published by Belgrano et al. in 2019 reported repeated use of limb perfusion, with 60% of complete response in the first session, and 41% and 59% in subsequent sessions²². In this study, no multiple perfusions were carried out in a single patient.

In the present study, nine patients died at 18 months, and although to date no locoregional treatment for melanoma in-transit metastases has had an impact on overall survival¹, in this publication, survival is lower than that reported in the literature, which is 29% at 5 years³, possibly because acral and nodular lentiginous melanomas, whose tumor biology is more aggressive, are predominant^{14,23}.

Local recurrences are described to range between 40% and 50% 6 months after treatment^{1,3,24}, which are similar results to those found in patients with surgically-resected in-transit metastases. In this study, local recurrences occurred in 36.3% of patients during a median follow-up of 18 months; it is not possible to make a comparison at 6 months because data are not available. In a systematic review of randomized studies, no benefit was found with the use of prophylactic ILP²⁰.

The toxicity found in our series was similar to that described in the literature^{1,15,25}. A study published in Brazil in 2012² reported ILP toxicity in 46 patients diagnosed with melanoma; perfusion was performed with Melphalan, as in our series, and grade I toxicity was found to be 2%; grade II, 78%; grade III, 7%; grade IV, 5%; and grade V, 9%. In our study, grade II toxicity was also the most common, although there were no grade IV or V toxicities. According to different publications, the factors associated with higher toxic-

ity are temperature greater than or equal to 40 °C, acidic pH, hypoxia and hematocrit values lower than 20%^{3,26}.

ILP performance prevented limb amputation in all patients. In the literature, limb salvage rates are similar at around 97%³, and in a systematic review of randomized trials, amputation rate was 0.005%²⁰.

Studies like ours use Melphalan as medication for ILP, but tumor necrosis factor alpha (TNF α) has also been used, which increases intra-tumor vascular permeability by improving cytostatic drug bioavailability. Recent investigations evaluated the use of the combination of TNF α and Melphalan, with complete disease responses being obtained in 80%^{3,24,27-30}, but the ACOSOG Z0020²⁵ trial compared the effectiveness of TNF α plus Melphalan with Melphalan alone in patients with melanoma of the extremities and in-transit metastases. After an interim analysis in 2004, terminating the study was decided because there was no difference in response between both groups, but there was higher toxicity in the group of patients who received TNF α . However, many centers continue to recommend the use of TNF alpha associated with Melphalan in patients with large tumor volumes, or in patients who are candidates for a second perfusion³. Either way, this drug is not available in Colombia.

For future studies, defining the role of ILP in current era of immunotherapy is going to be of great importance. Loco-regional treatments can cause tumor immunogenic cell death and other favorable immunogenic events. Some studies in animal models, in addition to anecdotal data from human cases, suggest that regional therapies may have an abscopal effect distant from the treated region³¹. Melanoma exposed to Melphalan stimulates the expansion of CD 16+ monocytes and activates cytotoxic T cells that contribute to ILP antitumor efficacy. In addition, tumor cell death during ILP results in the release of large amounts of antigens that increase T-cells response³². Patients in the present study were treated when immunotherapy and targeted therapies were not yet available. The surprising improvement in melanoma patients' outcomes with these systemic therapies in recent years makes for the study of the abscopal effect of local therapies in combination with systemic therapies to be essential³³. Some studies conclude that the use of regional treatments such as ILP and isolated infusion in melanoma patients with ITM remains a useful strategy because they have a good and lasting response³⁴.

It was not possible for patient quality of life to be assessed because data on the subject were not found

in the medical records, which is one of the most common limitations of retrospective studies. A study published in 2019 reported that patients with in-transit metastases undergo multiple surgical resections with high morbidity and early relapses; in contrast, with limb perfusion treatment, patients and physicians reported a sustained decrease in tumor burden and, therefore, better quality of life³⁵.

The limitations of this study include that it is a small cohort of patients with retrospectively collected data, which is why it was not possible to find the number of in-transit metastases in the medical records. In addition, it was not possible to find data on some variables such as the surgical time of two patients and Breslow depth of one patient. There is no comparative group, which means that comparisons are to be made with other articles in the literature; in addition, the study was carried out at a time in which immunotherapy was not available, which makes for the interaction of both these treatments impossible to be evaluated.

Conclusion

ILP remains an important tool for the treatment of unresectable melanoma of the extremities with in-transit metastases for improving functionality and quality of life of patients. Therapeutic ILP cannot be expected to improve survival or slow systemic disease progression. The response may change according to the melanoma clinical type, and is lower in patients with acral lentiginous melanoma and nodular melanoma when comparing this study with the world literature, although due to the low power of this study and the lack of a comparison group, the association between the response to ILP and melanoma clinical type cannot be defined. Finally, patients with locally-advanced unresectable melanoma are candidates for immunotherapy, and it is unlikely for a regional therapy to be used alone in patients with extensive disease; this opens the door for ILP combined use, which provides a rapid response in patients with high tumor burden in the extremities, associated with immunotherapy, which improves survival. Further studies are needed in order to define if both these therapies in combination can improve the outcomes for these patients.

Funding

This research has not received specific support from public or commercial sector agencies, or non-profit entities.

Conflicts of interest

The authors have no conflicts of interest to declare.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments have been performed on humans or animals for this research.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained informed consent from the patients and/or subjects referred to in the article. This document is in the possession of the corresponding author.

References

- Moreno-Ramirez D, de la Cruz-Merino L, Ferrandiz L, Villegas-Portero R, Nieto-García A. Isolated Limb Perfusion for Malignant Melanoma: Systematic Review on Effectiveness and Safety. *Oncologist*. 2010;15:416-427. doi:10.1634/theoncologist.2009-0325
- Neto D, Oliveira IF, Bertolli IIE, et al. Isolated limb perfusion with hyperthermia and chemotherapy : predictive factors for regional toxicity. 2012;67(3):237-241. doi:10.6061/clinics/2012(03)06
- Kroon BBR, Noorda EM, Vroenenraets BC, Slooten GW Van, Nieweg OE. Isolated Limb Perfusion for Melanoma. 2008;17:785-794. doi:10.1016/j.soc.2008.04.005
- Creech O, Krementz E, Ryan RF, Winblad JN. Chemotherapy of Cancer : Regional Perfusion Utilizing an Extracorporeal Circuit. *Ann Surg*. 1958;148(4):616-632.
- Colomb FM, Postel AH, Hall AB, Gumport SL, Cox KR, C WJ. Chemotherapy of Human Cancer by Regional Perfusion. *Cancer*. 1962;15(4):828-845.
- Pavan Pasin V, Fernandes De Oliveira A, Oliveira Santos ID de A, Masako Ferreira L. Hyperthermic Isolated Limb Perfusion in the Treatment of In-Transit Melanoma Metastases Victor. *Rev Col Bras Cir*. 2008;35(3):159-161.
- Wieberdink J, Benckhuysen C, Braat R, Van Slooten E, Olthuis A. Dosimetry in Isolation Perfusion of the Limbs by Assessment of Perfused Tissue Volume and Grading of Toxic Tissue Reactions. *Eur J Cancer Clin Oncol*. 1982;18(10):905-910.
- Erdei E, Torres SM. A new understanding in the epidemiology of melanoma. *Expert Rev Anticancer Ther*. 2010;10(11):1811-1823.
- Pozzobon F, Acosta A, Carreño A, Fierro E. Características del melanoma cutáneo primario en el Instituto Nacional de Cancerología 2006-2010. *Rev Colomb Cancerol*. 2013;17(3):111-118.
- Ferrari Junior NM, Muller H, Ribeiro M, Maia M, Sanchez Junior JA. Cutaneous melanoma : descriptive epidemiological study. *Sao Paulo Med J*. 2008;126(1):41-47.
- Gutiérrez C, Alarcón E, Valle R, Carderón G. Epidemiología del melanoma maligno en el Instituto Nacional de Enfermedades Neoplásicas, Perú, 2000-2004. *Folia Dermatol Peru*. 2007;18(1):23-27.
- Pozzobon FC, Acosta AE. Epidemiological profile of primary cutaneous melanoma over a 15-year period at a private skin cancer center in Colombia. *Rev Salud Pública*. 2018;20(2):226-231.
- Sortino-Rachou AM, Curado MP, Canela M de C. Cutaneous melanoma in Latin America: a population-based descriptive study. *Cad Saude Publica*. 2011;27(3):565-572. doi:10.1590/S0102-311X2011000300016
- Colmenares Roldán LM, Velásquez Lopera M, Vargas Suaza GA. Melanoma lentiginoso acral : una variante de melanoma maligno de especial interés en Colombia. *latreia*. 2008;21(4):386-397.
- Duarte C, García M, Lehmann C, Ricardo S, Manrique J. Perfusión aislada de extremidades. Experiencia inicial del Instituto Nacional de Cancerología, 2007-2008. *Rev Colomb Cancerol*. 2011;15(2):67-74.
- Fields RC, Fleming MD, Gastman B, et al. NCCN Melanoma Guidelines 2017. 2017;1.

17. Fink L, Amelio J, Harries M, Lebbe C, Livingstone E, Flinois A. Making decisions on resectability and injectability status of lesions in the management of advanced melanoma in Germany, France and the UK. *Eur J Dermatol.* 2016;26(5):477-486. doi:10.1684/ejd.2016.2821
18. Grünhagen DJ, de Wilt JHW, van Geel AN, Eggermont AMM. Isolated limb perfusion for melanoma patients—a review of its indications and the role of tumour necrosis factor- α . *Eur J Surg Oncol.* 2006;32(4):371-380. doi:10.1016/j.ejso.2006.01.015
19. Weitman ES, Zager JS. Regional therapies for locoregionally advanced and unresectable melanoma. *Clin Exp Metastasis.* 2018;0(0):0. doi:10.1007/s10585-018-9890-1
20. Lens MB, Dawes M. Review Isolated limb perfusion with melphalan in the treatment of malignant melanoma of the extremities : a systematic review of randomised controlled trials. *Lancet Oncol.* 2003;44:359-364.
21. Li Y, Mcclay EF. Systemic Chemotherapy for the Treatment of Metastatic Melanoma. *Semin Oncol.* 2002;29(5):413-426. doi:10.1053/sonc.2002.35237
22. Belgrano V, Pettersson J, Nilsson JA, Mattsson J, Katsarelias D, Olofsson Bagge R. Response and toxicity of repeated isolated limb perfusion (re-ILP) for patients with in-transit metastases of malignant melanoma. *Ann Surg Oncol.* 2019;26(4):1055-1062. doi:10.1245/s10434-018-07143-4
23. Duarte C, López H. Melanoma acral lentiginoso, revision bibliográfica. *Rev Colomb Cirugía.* 2014;29(2):155-166.
24. Mckinnon JG. Palliation by Perfusion : Is it Worth it ? *J Surg Oncol.* 2005;91:217-218. doi:10.1002/jso.20316
25. Cornett WR, Mccall LM, Petersen RP, et al. Randomized Multicenter Trial of Hyperthermic Isolated Limb Perfusion With Melphalan Alone Compared With Melphalan Plus Tumor Necrosis Factor : American College of Surgeons Oncology Group Trial Z0020. *J Clin Oncol.* 2006;24(25):4196-4201. doi:10.1200/JCO.2005.05.5152
26. Klaase JM, Kroon BBR, Van Geel BN, Eggermont AMM, Franklin HR, Hart GAM. Patient and treatment related factors associated with acute regional toxicity after isolated perfusion for melanoma of the extremities. *Am J Surg.* 1994;167:618-620.
27. Deroose JP, Eggermont AMM, Geel AN Van, Wilt JHW De, Burger JWA, Verhoef C. 20 Years Experience of TNF-Based Isolated Limb Perfusion for In-Transit Melanoma Metastases : TNF Dose Matters. *Ann Surg Oncol.* 2012;19:627-635. doi:10.1245/s10434-011-2030-7
28. Grünhagen D, de Wilt JHW, Van Geel AN, Eggermont AMM. Isolated limb perfusion for melanoma patients — a review of its indications and the role of tumour necrosis factor- α . *EJSO.* 2006;32:371-380. doi:10.1016/j.ejso.2006.01.015
29. Fraker DL, Alexander HR, Andrich M, Rosenberg SA. Treatment of patients with melanoma of the extremity using hyperthermic isolated limb perfusion with melphalan, tumor necrosis factor, and interferon gamma: results of a tumor necrosis factor dose-escalation study. *J Clin Oncol.* 1996;14(2):479-489. doi:10.1200/JCO.1996.14.2.479
30. Lienard D, Ewalenko P, Delmotte J, Renard N, Lejeune FJ. High-Dose Recombinant Tumor Necrosis Factor alpha in Combination With Interferon gamma and Melphalan in Isolation Perfusion of the Limbs for Melanoma and Sarcoma. *J Clin Oncol.* 1992;10(1):52-60.
31. Grünhagen BDJ, Verhoef C. Isolated Limb Perfusion for Stage III Melanoma : Does It Still Have a Role in the Present Era of Effective Systemic Therapy ? : Page 2 of 2. *Oncol J.* 2016;30(12):1045-1052.
32. Johansson J, Kiffin R, Andersson A, et al. Isolated Limb Perfusion With Melphalan Triggers Immune Activation in Melanoma Patients. *Front Oncol.* 2018;8(December):1-11. doi:10.3389/fonc.2018.00570
33. Read RL, Thompson JF. Managing in-transit melanoma metastases in the new era of effective systemic therapies for melanoma. *Expert Rev Clin Pharmacol.* 2019;12(12):1107-1119. doi:10.1080/17512433.2019.1689121
34. Teras J, Kroon HM, Thompson JF, et al. First Eastern European experience of isolated limb infusion for in-transit metastatic melanoma confined to the limb: is it still an effective treatment option in the modern era? *Eur J Surg Oncol.* 2020;46(2):272-276. doi:10.1016/j.ejso.2019.10.039
35. Ekenberg M, Wesslau H, Bagge RO, Engström M. Patient experiences with isolated limb perfusion for malignant melanoma – a qualitative study. *Eur J Oncol Nurs.* 2019;43:101672. doi:10.1016/j.ejon.2019.101672